

Application No. 09/523,776
Amendment dated September 28, 2007
Reply to Office Action of May 29, 2007

3

Docket No.: 49632(71699)

REMARKS

Claims 48, 51 and 55 are pending. Applicant makes these amendments without prejudice to pursuing the original subject matter of this application in a later filed application claiming benefit of the instant application, including without prejudice to any determination of equivalents of the claimed subject matter. Support for these amendments appears throughout the specification and claims as filed. No new matter is introduced by these amendments.

Rejection under 35 U.S.C. § 103(a)

Claims 45-54 are rejected as unpatentable over Herron (US Patent 4,764,521) in view of Rubenstein (IDS, CJ) and Rephaeli et al. (US Patent 5,939,455). Applicant traverses. Applicant reiterates the arguments made in the previously filed responses.

Particularly in response to paragraph 10 at page 4 of the Action, it is asserted in the Action that the 132 declaration by Dr. Zeitlin was not found to be persuasive to overcome the rejection based upon Herron in view of Rubenstein et al. and Rephaeli et al. or Faller. It is asserted that the claims are not commensurate in scope with the evidence of unexpected results, and more specifically that it has not been established that a correlation exists between the superior property shown and treatment of cystic fibrosis. Applicant traverses.

Applicant submits a review article (Zeitlin, *N. Engl. J. Med.* 351:6 pp. 606-608 (2004)) that describes the role of the $\Delta F508$ allele in patients with cystic fibrosis. The $\Delta F508$ mutation results in misprocessing of the CFTR chloride channel in the endoplasmic reticulum and subsequent degradation of the protein. The absence of CFTR on the cell membrane is associated with chloride and sodium imbalance (see Zeitlin, page 607 and Figure 1B). An agent, in this case curcumin, that restores trafficking of the $\Delta F508$ CFTR to the cell membrane results in increased chloride channel function and amelioration of the symptoms of cystic fibrosis (see Zeitlin, page

Application No. 09/523,776
Amendment dated September 28, 2007
Reply to Office Action of May 29, 2007

4

Docket No.: 49632(71699)

607 and Figure 1C). Thus, contrary to the assertion in the Action, it is in fact well established that promoting the trafficking of functional $\Delta F508$ -CFTR to the cell surface is relevant to ameliorating the symptoms of cystic fibrosis.

Applicant reiterates that their claimed subject matter has unexpectedly superior activity relative to cited art compounds cinnamic acid and 4-PBA. As presented previously, cells treated with trans-SAA produced increased amounts of both the immature and the mature forms of $\Delta F508$ -CFTR, consistent with an increase in CFTR production. These results indicate that trans-SAA is surprisingly effective in promoting the trafficking of functional $\Delta F508$ -CFTR to the cell surface relative to cinnamic acid and 4-PBA; and the unexpectedly superior activity of 4-phenyl- $\Delta 3$ -transbutenoic acid in promoting trafficking of $\Delta F508$ CFTR to the cell membrane is significant for treatment of cystic fibrosis, because trafficking of $\Delta F508$ CFTR to the cell surface increases chloride channel function. Based on the foregoing, Applicant respectfully submits that the rejection is overcome and requests withdrawal of the rejection.

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to pass this application to issue. Should any of the claims not be found to be in condition for allowance, the Examiner is requested to call Applicants' undersigned representative to discuss the application. Applicants thank the Examiner in advance for this courtesy.

Application No. 09/523,776
Amendment dated September 28, 2007
Reply to Office Action of May 29, 2007

5

Docket No.: 48632(71699)

The Director is hereby authorized to charge or credit any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 04-1105, under Order No. (71699) 49632.

Dated: September 28, 2007

Respectfully submitted,

By


Jeffrey D. Hsi

Registration No.: 40,024

EDWARDS ANGELL PALMER & DODGE
LLP

P.O. Box 55874

Boston, Massachusetts 02205

(617) 517-5569

Attorneys/Agents For Applicant

CLINICAL IMPLICATIONS OF BASIC RESEARCH

Can Curcumin Cure Cystic Fibrosis?

Pamela Zeitlin, M.D., Ph.D.

Cystic fibrosis, a progressive and ultimately fatal inherited disorder caused by a mutant cystic fibrosis transmembrane conductance regulator (CFTR) gene, has mobilized the government, charitable foundations, the biotechnology industry, and academia to work together to accelerate the development of drugs to combat the disease. The tools of molecular biology have facilitated the entry of about two dozen drugs in the developmental pipeline, any one of which, if successful, could halt the progression of the disease. As recently reported by Egan and colleagues,¹ the newest therapeutic candidate is curcumin, a so-called nutraceutical agent that is a mixture of compounds derived from the curry spice turmeric. Curcumin has been on the shelves of health food stores for some time and has been touted as an antioxidant, antiviral, antiinflammatory, anticancer, and cholesterol-lowering herbal supplement.

What led Egan et al. to study curcumin as a treatment for cystic fibrosis? They recognized that the most common cause of cystic fibrosis — the $\Delta F508$ mutation — results in a CFTR protein that is misprocessed in the endoplasmic reticulum — it is snagged by a chaperone protein and targeted for degradation, instead of making its way to the plasma membrane and forming a chloride channel. The absence of CFTR from the luminal-cell surface that lines the respiratory tract is associated with a deficit in chloride conductance regulated by cyclic AMP (cAMP) and a compensatory influx of sodium ions into the cell, with a consequent high sodium potential across the plasma membrane (Fig. 1). This promotes dehydration of the luminal environment and allows bacterial invasion and inflammation.

Because some chaperone proteins are dependent on high calcium levels, the authors reasoned that reducing the calcium levels in the endoplasmic reticulum might liberate the mutant CFTR, increasing the odds of its reaching the cell surface (Fig. 1). They had previously shown that curcumin inhibits a calcium pump (called sarcoplasmic reticulum Ca-ATPase) in the endoplasmic reticulum and that,

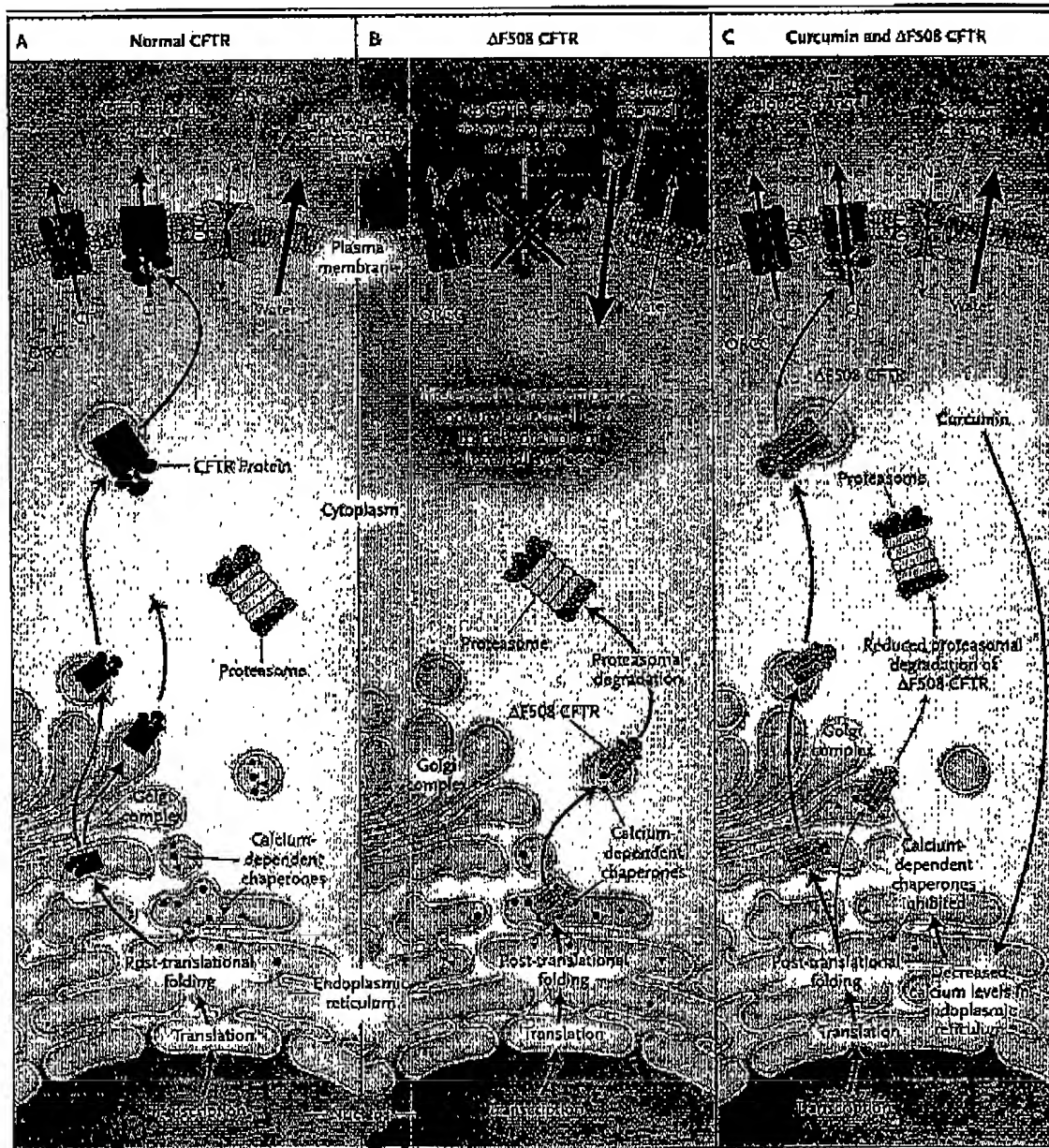
unlike some other inhibitors of this pump, curcumin has a low level of toxicity.

Egan et al. administered curcumin by oral gavage to mice engineered to express only the $\Delta F508$ allele.¹ This mutant mouse expresses the cystic fibrosis defect primarily in the gastrointestinal tract and rarely lives beyond four weeks. Treated mice had dramatically increased rates of survival and normal cAMP-mediated chloride transport across the nasal and gastrointestinal epithelia. In addition, the transepithelial sodium potential was reduced, suggesting that sufficient $\Delta F508$ protein reached the plasma membrane. Although the authors hypothesized that reduced calcium levels in the endoplasmic reticulum would ameliorate the effects of the $\Delta F508$ mutation by interfering with the function of the chaperone protein, the mechanism through which

Figure 1 (facing page). Curcumin, Calcium, and Cystic Fibrosis.

An important chloride channel in the plasma membrane is composed of cystic fibrosis transmembrane conductance regulator (CFTR) protein — the protein that is mutant in those with cystic fibrosis. The efflux of chloride ions into the lumen of the respiratory airways is coupled with the influx of sodium ions through the epithelial sodium channel, resulting in an ionic gradient across the plasma membrane and the movement of water from the cell into the lumen (Panel A). Wider black arrows indicate greater volume. Because the channels are coordinated, the activity of one affects the other. In patients with cystic fibrosis caused by the common $\Delta F508$ CFTR mutation, the mutant, misfolded CFTR protein is snagged by a chaperone protein and undergoes proteasomal degradation before it can reach the chloride channel. This results in higher sodium absorption from the lumen and dehydration of the luminal surface (Panel B). When Egan et al. administered the nutraceutical agent curcumin to mice that were homozygous for the mutant $\Delta F508$ allele, CFTR escaped degradation and appeared on the cell surface, the function of the chloride channel was restored, and the symptoms of cystic fibrosis were ameliorated (Panel C). Curcumin lowers calcium levels in the endoplasmic reticulum. These findings indicate a strategy to circumvent a proteasomal fate for $\Delta F508$ CFTR. ORCC denotes outwardly rectifying chloride channel.

CLINICAL IMPLICATIONS OF BASIC RESEARCH



CLINICAL IMPLICATIONS OF BASIC RESEARCH

curcumin counters these effects has yet to be determined.

Has anything like this effect of curcumin been seen before? The butyrate class of compounds effects a similar restoration of $\Delta F508$ processing, transport, and function in vitro and in vivo,² although 4-phenylbutyrate did not lower the sodium potential in humans (or in the mutant mice studied by Egan et al.¹). Neither curcumin nor 4-phenylbutyrate is expected to alter the chloride conductance of $\Delta F508$ CFTR. The isoflavonoid class of molecules, including genistein, directly stimulates cAMP-mediated chloride transport in the $\Delta F508$ mouse model and in another mouse model of cystic fibrosis.³ Genistein, a phytoestrogen, is found in high levels in soy products such as tofu and is marketed as a nutraceutical agent for the chemoprevention of breast and prostate cancers, cardiovascular disease, and postmenopausal symptoms. The repressive effect of some CFTR premature stop mutations on the expression of functional CFTR channels in the nasal epithelium of patients can be countered by exposure to certain aminoglycosides.⁴ This mechanism seems to work by interfering with the synthesis of the CFTR protein; the ribosome is thought to skip over the unexpected stop codon to produce a full-length, normal CFTR protein. Agonists of non-CFTR chloride channels can effect transmembrane transport of chloride into the lumen in vitro and in vivo.⁵ Whether any of these approaches will be sufficient to halt or reverse the decline in lung function in patients with cystic fibrosis has yet to be determined.

The next step is to test the nutraceutical of curcumin in a phase 1, dose-escalation and safety trial in patients with cystic fibrosis. Such studies should

identify the active molecules and assess the absorption of the agent by the gastrointestinal tract, as well as the metabolism, safety, and duration of effect of curcumin. Physicians should counsel patients with cystic fibrosis to refrain from rushing to the health food store to buy curcumin, because inadequate or excessive doses of the agent could do more harm than good and could discourage the study of a potentially useful class of therapeutic agents. Instead, eligible patients should consider participating in placebo-controlled clinical trials of curcumin and thus speed up the movement of this compound through the developmental pipeline. On a more philosophical note, the study by Egan et al.¹ is another testament to the pharmacogenetic approach: small-molecule pharmacotherapy tailored to a specific genotype (in this case, the $\Delta F508$ mutation) is rapidly becoming a reality for patients with cystic fibrosis and other inherited disorders of protein function.

From the Division of Pediatric Respiratory Sciences, Johns Hopkins School of Medicine, Baltimore.

1. Egan ME, Pearson M, Weiner SA, et al. Curcumin, a major constituent of turmeric, corrects cystic fibrosis defects. *Science* 2004; 304:600-2.
2. Zeitlin PL, Dhanar-West M, Rubenstein AC, Boyle MP, Lee CK, Brass-Barnet L. Evidence of CFTR function in cystic fibrosis after systemic administration of 4-phenylbutyrate. *Mol Ther* 2002;6:119-26.
3. Illek BL, Zhang L, Lewis NC, Moss RB, Dong JY, Fischer H. Defective function of the cystic fibrosis-causing missense mutation G551D is recovered by genistein. *Am J Physiol* 1999;277:C833-C839.
4. Wilschanski M, Yahav Y, Yancov Y, et al. Gentamicin-induced correction of CFTR function in patients with cystic fibrosis and CFTR stop mutations. *N Engl J Med* 2003;349:1433-41.
5. Yernool BR, Sabater JR, Davis CW, et al. Pharmacology of IN337217 [P(1)-(uridine 5')-P(4)-(2'-deoxycytidine 5')tetraphosphate, tetrasodium salt], a next-generation P2Y₂ receptor agonist for the treatment of cystic fibrosis. *J Pharmacol Exp Ther* 2002;302: 871-80.

Copyright © 2004 Massachusetts Medical Society.